Kidney International, Vol. 50 (1996), pp. 1659–1662

HIV-1 survival kinetics in peritoneal dialysis effluent

HOMAYOON FARZADEGAN, DAVID FORD, MICHELE MALAN, BETH MASTERS, and PAUL J. SCHEEL, JR.

Department of Medicine, The Johns Hopkins University School of Medicine, and the Department of Epidemiology, The Johns Hopkins School of Public Health, Baltimore, Maryland, USA

HIV-1 survival kinetics in peritoneal dialysis effluent. Viable and potentially infectious HIV-1 has been recovered from the peritoneal dialysis effluent (PDE) of patients with end-stage renal disease (ESRD) who are infected with the human immunodeficiency virus (HIV). No information had previously been available as to how long HIV-1 could survive in this environment, and no data were available as to how long HIV-1 could survive on peritoneal dialysis exchange tubing (PDET). Therefore, this study was designed to answer these questions. HIV-1 Mn was added to PDE and allowed to incubate at room temperature for 0 to 14 days. Following centrifugation, the cellular component of the PDE mixture was placed in co-culture with peripheral blood mononuclear cells (PBMC) from HIV negative donors. Aliquots from the co-cultures were removed after 14 days and assayed for the HIV-1-P24 antigen. High levels of HIV P24 antigen were recovered up to and including seven days of room temperature incubation. HIV could not be recovered from PDE that had been incubated at room temperature for 10 to 14 days. Ten milliters of HIV-PDE mixture was placed within PDET and incubated at room temperature for 10 minutes. The solution was then removed by gravity drainage. After drying times of 0 to 168 hours, the tubing was flushed with HIV culture medium and placed in co-culture with PBMCs from HIV negative donors. The culture supernatant was assayed for the HIV-1 P24 antigen as a marker of viral replication. High levels of HIV-1 P24 antigen were recovered from the PDET wash for up to and including 48 hours of drying time. No viable virus could be detected for drying times of between 72 and 168 hours. To determine if common disinfectants found in the dialysis unit could inactivate HIV, dilutions of Amukin® 50% and household bleach were prepared at final concentrations ranging from 1:32 to 1:2048. These disinfectant solutions were incubated with PDE containing HIV for 10 minutes. The cellular fraction of the PDE was isolated by centrifugation, washed, and placed in co-cultures with peripheral blood mononuclear cells. HIV P24 antigen levels were assayed every three days for 28 days. Amukin® 50% and a 10% household bleach solution were effective in killing HIV in PDE at dilutions up to and including 1:512. These results indicate that HIV can survive in PDE at room temperature for up to seven days. HIV can survive on peritoneal dialysis exchange tubing for up to 48 hours. Final dilutions of 1:512 Amukin® 50% and 10% household bleach, after 10 minutes of exposure, are effective viricidal agents in disinfecting PDE.

Peritoneal dialysis has been shown to be an appropriate alternative to hemodialysis in the management of end-stage renal disease (ESRD) in patients infected with the human immunodeficiency virus (HIV). Early reports focused on patient survival and hemodialysis and peritoneal dialysis were found to be similar [1–3].

Received for publication April 4, 1996
and in revised form June 6, 1996
Accepted for publication June 6, 1996

Recently, attention has been focused on the potential infectious nature of peritoneal dialysis effluent (PDE) in individuals infected with HIV. Williams, Swann and Cunningham were the first to describe the presence of the antibody to HIV-1 in PDE in 1986 [4]. Subsequent studies by Breyer et al, Scheel et al, and Goffin et al confirmed the presence of viable HIV in PDE utilizing co-culture systems, but provided no data on the survival of the virus in this environment [5–7].

This study was designed to determine: (1) the length of time that HIV-1 could remain viable in PDE; (2) the length of time that HIV-1 could remain viable on peritoneal dialysis exchange tubing (PDET); and (3) the dynamics of viricidal effectiveness of common disinfectants found in the dialysis unit, that is, a solution of household bleach or Amukin® to disinfect PDE containing HIV.

Methods

Peritoneal dialysis effluent

Peritoneal dialysis effluent (PDE) was obtained from donors who were serologically antibody negative for HIV-1. Culture of the donor's PDE, by methods previously described, were also negative for growth of HIV-1 [7]. Fresh samples of PDE originally containing 1.25%, 2.5% or 4.25% dextrose were obtained and used immediately for experimental purposes.

HIV culture media

HIV culture medium consisted of RPMI medium containing 20% fetal bovine serum, 2% interleukin 2, 1% sodium bicarbonate, 2 mM L-glutamine, and 4 μg/ml of phytohemagglutinin-P (PHA-P).

HIV-PDE growth experiment

One hundred $TCID_{50}$ of HIV-1 Mn was added to PDE that originally contained either 1.25%, 2.5%, or 4.25% dextrose, or to HIV-RPMI culture medium. The mixtures were incubated at room temperature, aliquots were removed at 0 and at 3, 7, 10, and 14 days, and placed in a co-culture system with PHA-P stimulated peripheral blood mononuclear cells (PBMCs) that were obtained from HIV negative donors. After 14 days of culture at 37°C, aliquots were removed and assayed for HIV-1 P24 antigen using an ELISA technique (Abbott Laboratories, North Chicago, IL, USA). Viral replication was considered present if > 30 pg/μl of HIV-1 P24 antigen was present [8].

Peritoneal dialysis exchange tubing experiment

Sterile peritoneal dialysis exchange tubing was supplied by Fresenius, USA (Walnut Creek, CA, USA). One hundred $TCID_{50}$

Table 1. Survival of HIV-1 in RPMI and PDE containing various concentrations of dextrose

Days of HIV-1 suspensions in various media	HIV-1 P24 value[b] after 14 days of coculture (+ or − culture)			
	1.5% dex.	2.5% dex.	4.25% dex.	RPMI
0[c]	82.2 (+)	112.8 (+)	95.2 (+)	95.9 (+)
3	41.7 (+)	26.0 (+)	43.5 (+)	0.7 (+)
7	7.9 (+)	16 (+)	21.5 (+)	0.2 (−)
10	0.2 (−)	0.2 (−)	0.2 (−)	0.2 (−)
14	0.2 (−)	0.2 (−)	0.2 (−)	0.2 (−)

[a] RPMI or PDE + (1.5, 2.5, and 4.25% dextrose)
[b] The HIV-1 P24 antigen concentration × 1000 pg/μl
[c] Control culture

Table 2. Recovery of HIV-1 from peritoneal dialysis exchange tubing (PDET) after *in vitro* contamination with 100 $TCID_{50}$ of HIV-1 Mn

Drying time hours	Weeks in culture			
	Week one		Week two	
	P24 1st Test*	P24 2nd Test*	1st Test*	2nd Test*
0	131 (+)	131 (+)	451 (+)	Bleached
2	168 (+)	441 (+)	480 (+)	490 (+)
4	102 (+)	194 (+)	429 (+)	439 (+)
8	107 (+)	307 (+)	480 (+)	439 (+)
24	130 (+)	80 (+)	272 (+)	439 (+)
48	113 (+)	123 (+)	357 (+)	Bleached
72	113 (+)	57 (+)	30 (−)	10 (−)
168	61 (+)	23 (−)	9 (−)	8 (−)

Coculture technique was used to isolate HIV-1 after one to 168 hours of drying time in tubes pg/μl of HIV P24 antigen (+ or − culture). Virus free HIV culture media served as negative control.

Test is defined as the HIV-P24 antigen capture technique. The values given are HIV-1 P24 antigen in pg/μl of culture fluid. Plus (+) equals HIV-P24 > 30 pg/μl and minus (−) equals HIV-P24 < 30 pg/μl.

of HIV-1 Mn was suspended in HIV RPMI culture medium and mixed with 10 ml of PDE. The HIV-1 containing PDE mixture was placed in the lumen of the PDET, the ends of the tubing were clamped, and placed on a rocker platform for one hour at room temperature. The fluid within the tubing was drained by gravity and disinfected by bleach. The tubing was flushed immediately with 10 ml of RPMI culture media containing 1.0×10^6 PHA-P stimulated PBMCs from negative donors. This wash solution was placed in a co-culture flask and incubated to 37° for four weeks. This protocol was repeated for 2, 4, 8, 24, 48, 72, and 168 hours of room temperature incubation post-gravity drainage.

Aliquots were removed from the cultures twice weekly for four weeks and assayed for the HIV-1 P24 antigen using an ELISA technique (Abbott Laboratories). Viral replication was considered present if > 30 pg/μl of HIV-P24 antigen was present in culture fluid and serial analysis of subsequent time points revealed rising quantities of P24 antigen. All cultures were disinfected and discarded after 28 days.

Disinfection experiments

One hundred $TCID_{50}$ of HIV-1 Mn in HIV culture media was added to PDE originally containing 2.5% dextrose. A 10% solution of household bleach or undiluted Amukin™ was added to the PDE at final dilutions of 1:32, 1:128, 1:512, or 1:2,048. The disinfectant and PDE were incubated at room temperature for 10 minutes. The solution was centrifuged for one hour at 19,000 rpm at 23°C to pellet down HIV-1 from suspension. This step was performed to remove the disinfectants. The pellet was resuspended in HIV-1 RPMI culture media and cultured immediately with PBMCs from HIV negative donors. Aliquots were removed from the culture every three days for 28 days and assayed for the HIV-1 P24 antigen. A positive culture was defined if > 30 pg/μl of P24 antigen was present and subsequent serial analysis of culture fluid revealed rising quantities of P24 antigen.

Results

Peritoneal dialysis effluent

As shown in Table 1, positive cultures for HIV-1 were obtained for samples taken from the RPMI media and for all concentrations of dextrose in PDE (1.25%, 2.5%, and 4.25%) at time 0. This time point served as control. After three days of incubation at room temperature, there was evidence of viral replication in RPMI. However, significantly higher growth of HIV-1 was observed in all three PDE solutions. On Day 7, there was no evidence of HIV replication in RPMI culture media, but there were positive cultures obtained in all three concentrations of dextrose in PDE. HIV-1 growth was not observed in cultures performed on Days 10 and 14 for RPMI and all dextrose containing PDE solutions.

Peritoneal dialysis exchange tubing

As summarized in Table 2, HIV-1 was recovered from PDET in washes obtained immediately after incubation with solution containing 100 $TCID_{50}$ of HIV-1 Mn (drying time = 0). These cultures were positive for HIV-P24 antigen in the first week and reached high values within 14 days of co-culturing. Infectious HIV was also recovered from PDET for up to 48 hours after PDET were contaminated with 100 $TCID_{50}$ of HIV-1 Mn and left at room temperature to dry. PDET with drying times of 72, and 168 hours showed low (50 to 113 pg/ml) levels of HIV-P24 antigen in the first week of culture. Levels fell below the sensitivity of the assay at 14 days of follow up, indicating the absence of infectious HIV-1 in these PDET.

Disinfection experiments

As shown in Figure 1, the addition of Amukin™ 50% to final dilutions of 1:32, 1:128, and 1:512 resulted in falling concentrations of HIV-P24 antigen levels from Day 3 through Day 20 of co-culture, indicating viral death. The addition of Amukin™ 50% to PDE at a final concentration of 1:2,048 did not effect the growth of HIV in co-culture and the quantitative HIV-P24 antigen levels measured did not differ from PDE not containing disinfectant (control solution).

The disinfectant effect of a 10% bleach solution was similar to that demonstrated for Amukin™ 50%. Figure 2 shows decreasing levels of HIV-P24 antigen level in co-cultures where the PDE had been exposed to a 10% bleach solution further diluted to final dilutions of 1:32, 1:128, and 1:512 in the PDE HIV solution. These HIV-1 P24 antigen results indicate viral death. The quantitative P24 antigen levels obtained from the PDE exposed to a bleach solution at a concentration of 1:2,048 did not differ from the control values and were similar to those obtained for the 1:2,048 dilution obtained with Amukin™ 50%.

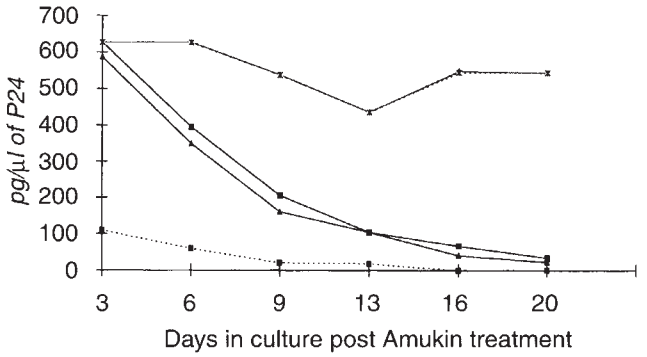

Fig. 1. *HIV-1 P24 antigen levels obtained from co-culture supernatants after 3 to 20 days of culture, following 10 minutes of disinfection with various concentrations of Amukin™ 50%.* Symbols are the 1:x ratio of Amukin™ to 100 TCID Mn/PDF solution: (◇) 0; (●) 32; (▲) 128; (■) 512, (-X-) 2048.

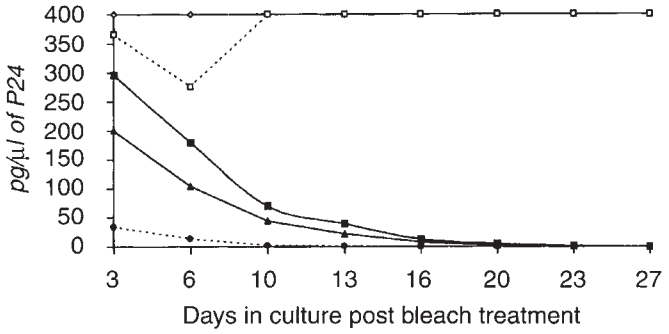

Fig. 2. *HIV-1 P24 antigen levels obtained from co-culture supernatants after 3 to 20 days of culture, following 10 minutes of disinfection with various concentrations of 10% bleach.* Symbols are the 1:x ratio of bleach to 100 TCID Mn/PDF solution: (◇) 0; (●) 32; (▲) 128; (■) 512, (□) 2048.

Discussion

The recovery of viable HIV-1 from peritoneal dialysis effluent obtained from individuals infected with this virus has been documented by several authors [5–7]. In each of these studies, the cellular component of "fresh" effluent was placed in a co-culture system and viral growth was documented in 75 to 100% of patients [5–7]. The ability to recover virus from the PDE did not correlate with the patient's blood CD4 count and the oral administration of antiviral agents, such as AZT, did not appear to effect the growth of HIV in the PDE [7]. Breyer and Harbison have subsequently reported that HIV-1 could be recovered from one patient's PDE after 72 hours of room temperature incubation, suggesting that HIV may survive longer than anticipated in this environment [9]. This study is the first to formally and extensively study the survival kinetics of HIV-1 in PDE and on PDET.

Our results indicate that HIV-1 can survive for three days at room temperature in RPMI culture media and PDE solutions containing various concentrations of dextrose. Cultures performed after three days showed that HIV-1 survived well in PDE solution (P24 values > 26,000 pg/μl), but showed marginal HIV-1 survival in RPMI culture media (P24 values 700 pg/μl). HIV-1 did not survive in RPMI after seven days of suspension at room temperature, while PDE containing various concentrations of dextrose supported HIV-1 under similar conditions. This finding suggests that PDE contains the appropriate conditions required to sustain HIV-1 and may provide a more suitable environment for growth than traditional cell-free HIV-1 culture media.

HIV-1 did not survive after 10 days suspension in PDE or RPMI, as all P24 values obtained from these cultures were similar to background values for the inoculum of HIV-1 used in the experiment (200 pg/μl of HIV P24 antigen).

In vitro contamination of peritoneal dialysis exchange tubing with live HIV-1 at levels comparable to average infectious viral load of AIDS patients (< 200 CD4 levels) was used to determine the survival of HIV-1 on these tubes at room temperature. These experiments were designed to determine, for the first time, the maximum period of time that HIV-1 can survive the dry conditions within PDET. As shown in Table 2, HIV-1 was easily and rapidly recovered from the tubing after HIV-1 contaminated solutions were decanted and tubes were washed with RPMI solution and cultured immediately (drying time = 0). HIV-1 was also easily recovered from PDET after two, four, and eight hours of drying time. Most cultures from these tubes were positive within the first seven days of cultures, indicating that large quantities of viable and infectious HIV were present in the tubing contaminated with 100 $TCID_{50}$ of HIV-1 after two to eight hours of drying conditions. Tubing with 24 and 48 hours of drying time showed low levels of HIV-1 P24 antigen in the first week of culture, which reached high values within the second week of culture. This suggests that lower levels of infectious HIV survived after 24 or 48 hours of drying time. HIV-1 was not recovered from PDET after 72 to 168 hours or drying time. The low levels of HIV-1 P24 antigen recovered from culture supernatants during the first week of culture may reflect the background HIV-1 P24 in the tubing washes, presumably from dead HIV-1 virons. These findings indicate that HIV-1 can survive drying conditions within the CAPD tubing for up to 48 hours. These tubes must be autoclaved, or disinfected, with effective viricidal disinfectant to ensure viral destruction and to avoid a potential hazard for individuals handling this tubing.

The results of disinfection in these experiments, using a 10% bleach solution and Amukin™ 50% in 2.5% dextrose PDE solutions, showed that final dilutions of 1:32, 1:128, and 1:512 of either disinfectant effectively killed 100 $TCID_{50}$ of HIV-1 Mn in 10 minutes at room temperature. Common household bleach at 1:2048 final dilution was incapable of killing HIV, since the cultures performed on pelleted viruses resulted in off scale and positive P24 values after 10 days of culture. A similar observation was made with the disinfectant Amukin™ 50% where 1:2048 dilution of Amukin™ 50% showed off scale and positive cultures similar to the negative (no-bleach) control.

In summary, our studies showed that HIV-1 can survive in PDE for up to seven days at room temperature and can survive on peritoneal dialysis exchange tubing under dry conditions for up to 48 hours. Bleach and Amukin™ can effectively disinfect HIV-1 in dialysis fluids at final dilutions up to 1:512 when 100 TCID of HIV-1 is present in the peritoneal dialysis effluent. This viral load is thought to be the equivalent of HIV-1 found in PDE in the non-peritonitis state. It is possible in patients who have peritonitis, who have a larger cellular fraction in their PDE, that a higher level of disinfection will be required. Until further information is obtained, all patients and dialysis personnel should exercise universal precautions when handling and disposing of PDE. Patients and their caregivers, as well as dialysis personnel, should

be informed of the potential infectious nature of peritoneal dialysis fluid and peritoneal dialysis exchange tubing, and appropriate measures should be taken to dispose of these materials in a proper manner.

Acknowledgment

This work was supported in part by a research grant from Baxter Health Care, Renal Division, McGaw Park Illinois. This study was presented in part at the 1995 Annual Conference on Peritoneal Dialysis, Baltimore, Maryland.

Reprint requests to Paul J. Scheel, Jr., M.D., The Johns Hopkins University School of Medicine, Division of Nephrology, Suite 958, Ross Building, 720 Rutland Avenue, Baltimore, Maryland 21205, USA.

References

1. KIMMEL PL, UMANA WU, SIMMENS S, WATSON J, BOSCH J: Continuous ambulatory peritoneal dialysis and survival of HIV infected patients with end-stage renal disease. *Kidney Int* 44:373–378, 1993
2. TEBBE JA, RIGSBY MO, SELWYN P, BRENNAN N, KLISEN A, FINKELSTEIN F: Outcome of HIV infected patients on continuous ambulatory dialysis. *Kidney Int* 44:191–198, 1993
3. ORITZ C, MENESES R, JAFFE D, FERNANDEZ J, PEREZ G, BOURGOIGNIE JJ: Outcome of patients with human immunodeficiency virus on maintenance hemodialysis. *Kidney Int* 34:248–253, 1988
4. WILLIAMS PF, SWANN B, CUNNINGHAM K: Continuous ambulatory peritoneal dialysis fluid: Another fluid positive for HIV antibody? *Br Med J* 293:885, 1986
5. BREYER J, HARBISON MA: Isolation of human immunodeficiency virus from peritoneal dialysate. *Am J Kid Dis* 21:23–25, 1993
6. GOFFIN E, VANDERCAM B, BURTONBOY G: Detection of HIV antigen in peritoneal dialysis fluid. *Peritoneal Dial Int* 14:889, 1994
7. SCHEEL PJ, FARZADEGAN H, FORD D, MALAN M, WATSON A: Recovery of human immunodeficiency virus from peritoneal dialysis effluent. *J Am Soc Nephrol* 5:1926–1929, 1995
8. FARZADEGAN H, IMAGAWA D, GUPTA P, LE M, JACOBSON L, SAAH A, GROVIT K, RINALDO CR JR, POLK BF: The effect of fresh lymphocytes on increased sensitivity of HIV-1 isolation: A multi center study. *J Acquired Immune Def Syndromes* 3:981–986, 1990
9. BREYER J, HARBISON M: Isolation and quantitation of human immunodeficiency virus type I (HIV-1) from peritoneal dialysate: Implications for infection control. *Peritoneal Dial Int* 15:179–180, 1995